

CLAIMS

We claim:

1. A method for treating or preventing a condition affected by endocytosis or down-regulation of cell surface receptors, comprising administering to a patient in need thereof a therapeutically effective amount of a MARCH-protein antagonist or pharmaceutical composition thereof, wherein the antagonist inhibits MARCH-protein-mediated endocytosis or down-regulation of type I or type II cell surface receptors.

2. The method of claim 1, wherein the MARCH antagonist is selected from the group consisting of anti-MARCH-protein antibodies or epitope-binding fragments thereof, MARCH-specific antisense oligomers, proteins and polypeptides, small molecule MARCH inhibitors of MARCH-protein-mediated ubiquitination, and combinations thereof.

3. The method of claim, 2, wherein the MARCH-specific antisense oligonucleotide comprises a sequences of at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS:38, 40, 42, 44, 46, 48, 50 and SEQ ID NO:52, and complements thereof

4. The method of claim 1, wherein the MARCH-protein is selected from the group consisting of MARCH-I through MARCH-VII, and MARCH-VIII, corresponding to SEQ ID NOS:39 and 54, SEQ ID NOS:41 and 55, SEQ ID NOS:43 and 56, SEQ ID NOS:45 and 57, SEQ ID NOS:47 and 58, SEQ ID NOS:49 and 59, SEQ ID NOS:51 and 60, SEQ ID NOS:53 and 61, respectively.

5. The method of claim 1, wherein the MARCH-protein-mediated endocytosis of type I or type II cell surface receptors, involves a receptor selected from the group consisting of transferrin receptor, FAS (APO-1/CD95), MHC-I and other histocompatibility antigens, HLA-A2.1 or CD4, ICAM-1, B7.2, and combinations thereof.

6. The method of claim 1, wherein the condition is selected from the group consisting of leukemia and other cancers, mental retardation, thalassemia, autoimmune disease

and neurological disease.

7. A method for identifying test compounds having therapeutic activity for a condition affected by endocytosis of cell surface receptors, comprising:

(a) contacting a test compound with a MARCH-protein target/receptor and a functional
5 MARCH-protein wherein at least one of the proteins bears a detectable label;

(b) assaying any resulting MARCH-protein target/receptor:MARCH-protein complex for the presence of the label; and

(c) determining whether the test compound antagonizes binding of the MARCH-protein target/receptor to the MARCH-protein, whereby test compounds that antagonize said binding
10 are, at least in part, identified as therapeutic compounds.

8. The method of claim 7, wherein the MARCH-protein is selected from the group consisting of MARCH-I through MARCH-VII, and MARCH-VIII, corresponding to SEQ ID NOS:39 and 54, SEQ ID NOS:41 and 55, SEQ ID NOS:43 and 56, SEQ ID NOS:45 and 57, SEQ ID NOS:47 and 58, SEQ ID NOS:49 and 59, SEQ ID NOS:51 and 60, SEQ ID NOS:53 and
15 61, respectively.

9. The method of claim 7, wherein the MARCH-protein target/receptor is selected from the group consisting of transferrin receptor, FAS (APO-1/CD95), MHC-I and other histocompatibility antigens, HLA-A2.1 or CD4, ICAM-1, B7.2, and combinations thereof.

10. The method according to claim 7 wherein either the functional MARCH-protein
20 target/receptor or the functional MARCH-protein is immobilized onto a solid phase.

11. The method according to claim 7 wherein the MARCH-protein target/receptor or the MARCH-protein is labeled with a radiolabel, a fluorescent reporter or quencher moiety, an enzymic label that catalyzes a colorimetric or fluorometric change, or combinations thereof.

12. A method for identifying test compounds having therapeutic activity for a
25 condition affected by endocytosis of cell surface receptors, comprising:

(a) contacting a test compound with a cell expressing a functional MARCH-protein target/receptor and a MARCH-protein; and

(b) determining whether the test compound antagonizes at least one of MARCH-protein-mediated ubiquination activity, or MARCH-protein-mediated receptor endocytosis, whereby test compounds that antagonize at least one of such activity are identified as therapeutic compounds.

13. The method of claim 12 wherein the cell expresses a recombinant MARCH-protein target/receptor or a recombinant MARCH-protein.

14. The method of claim 12, wherein determination of antagonism of MARCH-protein-mediated activity is based on an assay selected from the group consisting of: inhibition of MARCH-protein-mediated endocytosis assays, receptor up- or down-regulation assays, cell motility assays, cell growth rate assays, apoptosis assays, ubiquination assays, and MARCH-protein-mediated or MARCH-protein target/receptor-mediated signal transduction assays.

15. The method of claim 14 wherein the MARCH-protein-mediated endocytosis assay is based on measurement of a cell-surface receptor selected from the group consisting of transferrin receptor, FAS (APO-1/CD95), MHC-I and other histocompatibility antigens, HLA-A2.1 or CD4, ICAM-1, B7.2, and combinations thereof.

16. The method of claim 14 wherein the MARCH-protein-mediated or MARCH-protein target/receptor-mediated signal transduction assay is based on measurement of cellular processes selected from the group consisting of phosphorylation or activation of an intracellular protein, organization of the actin cytoskeleton, gene transcription, lipid metabolism, vesicle trafficking, cellular transformation, cellular death, and combinations thereof, whereby test compounds that alter said cellular process, relative to those of control cells, are identified as therapeutic compounds.

17. The method according to claim 14, wherein the condition characterized by MARCH-protein-mediated cell growth, cell motility, or inhibition of endocytosis is cancer, mental retardation, or thalassemia.

18. A pharmaceutical composition, comprising an antagonist of a MARCH-protein,
5 and a pharmaceutically acceptable carrier or diluant.

19. The pharmaceutical composition of claim 18, wherein the MARCH-protein antagonist is a MARCH-specific antisense oligonucleotide comprising at least 12 contiguous nucleotides of a sequence selected from the group consisting of of SEQ ID NOS:38-45, and complements thereof.

10 20. An anti-MARCH-protein antibody.

21. The anti-MARCH-protein antibody of claim 20, wherein the antibody is a monoclonal antibody.

22. The anti-MARCH-protein monoclonal antibody of claim 21, wherein the monoclonal antibody is a single-chain antibody, chimeric antibody, humanized antibody or Fab
15 fragment.

23. A method for inhibiting endocytosis or down-regulation of cell-surface receptors, comprising contacting a cell, having a cell-surface receptor that is at least to some extent subject to MARCH-protein-mediated endocytosis, with a MARCH-protein antagonist, whereby said endocytosis or down-regulation is, at least to some extent, inhibited.

20 24. The method of claim 23, wherein the MARCH antagonist is selected from the group consisting of anti-MARCH-protein antibodies or epitope-binding fragments thereof, MARCH-specific antisense oligomers, proteins and polypeptides, small molecule MARCH inhibitors of MARCH-protein-mediated ubiquitination, and combinations thereof.

25 25. The method of claim, 24, wherein the MARCH-specific antisense oligonucleotide comprises a sequences of at least 12 contiguous nucleotides of a sequence selected from the group consisting of SEQ ID NOS:38, 40, 42, 44, 46, 48, 50 and SEQ ID NO:52, and

complements thereof

26. The method of claim 23, wherein the MARCH-protein is selected from the group consisting of MARCH-I through MARCH-VII, and MARCH-VIII, corresponding to SEQ ID NOS:39 and 54, SEQ ID NOS:41 and 55, SEQ ID NOS:43 and 56, SEQ ID NOS:45 and 57, 5. SEQ ID NOS:47 and 58, SEQ ID NOS:49 and 59, SEQ ID NOS:51 and 60, SEQ ID NOS:53 and 61, respectively.

27. The method of claim 23, wherein the MARCH-protein-mediated endocytosis or down-regulation of type I or type II cell surface receptors, involves a receptor selected from the group consisting of transferrin receptor, FAS (APO-1/CD95), MHC-I and other 10 histocompatibility antigens, HLA-A2.1 or CD4, ICAM-1, B7.2, and combinations thereof.